Aim: To investigate the effect of different antibiotics on the growth of bacteria, using aseptic technique to culture bacteria on agar plates.

Background Theory

- Bacteria reproduce rapidly and can be cultured (grown) on nutrient agar in petri dishes.
- Antibiotics kill or inhibit bacterial growth. Different antibiotics are effective against different bacteria.
- A zone of inhibition is the clear area around an antibiotic disc where bacteria have been killed/cannot grow.
- A larger zone of inhibition = the antibiotic is more effective against that bacterium.
- Aseptic technique prevents contamination of cultures with unwanted microorganisms.

★ Antibiotic resistance: if bacteria show no zone of inhibition, they may be resistant — a major public health concern.

Equipment

- Nutrient agar plates
- Bacterial culture (e.g. harmless E. coli strain)
- Antibiotic paper discs
- Sterile spreader (glass rod)
- Sterile forceps
- Inoculation loop
- Bunsen burner
- Adhesive tape
- Ruler (mm)
- Incubator (set to 25°C)

Aseptic Technique — Key Rules

- Work near a lit Bunsen burner to create an upward convection current that keeps microbes away.
- Flame all metal instruments (inoculation loop, spreader) and allow to cool before use.
- Never open agar plates fully — lift the lid at an angle (like a hinged door).
- Tape the lid on (but NOT all the way around — allow gas exchange).
- Incubate at 25°C, not body temperature — reduces risk of culturing harmful pathogens.

Method

1. Label the base of an agar plate with your name, date, bacteria used and antibiotic being tested.

2. Using aseptic technique, pipette 0.1 cm^3 of bacteria suspension onto the plate.

3. Sterilise a glass spreader by dipping in ethanol and passing through a Bunsen flame. Allow to cool briefly.

4. Spread the bacteria evenly across the whole agar surface — work quickly with the plate lid angled.

5. Using sterile forceps, place antibiotic discs onto the surface of the agar. Press gently.

6. Replace the lid. Secure with two pieces of tape (not all the way round).

7. Invert and incubate at 25°C for 24–48 hours.

8. After incubation, measure the diameter of any clear zones of inhibition around each disc (do not open the plate).

Variables

Independent variable	Type of antibiotic (different discs used)
Dependent variable	Diameter of the zone of inhibition (mm)
Controlled variables	Volume of bacteria spread, agar type, incubation temperature and time, same bacterial strain, disc diameter

Results Table

Antibiotic	Zone diameter — trial 1 (mm)	Zone diameter — trial 2 (mm)	Zone diameter — trial 3 (mm)	Mean diameter (mm)

Analysis

- Calculate the mean zone of inhibition diameter for each antibiotic.
- Larger zone = more effective antibiotic against this bacterium.
- A zone diameter of 0 mm = the bacterium is resistant to that antibiotic.

★ Calculate area of zone of inhibition: $A = \pi \times (d/2)^2$. This gives a more meaningful measure of effectiveness.

★ Discuss limitations: bacteria used in schools are safe strains and may not represent clinical pathogens.

Exam Tip: Incubate at 25°C, NOT 37°C (body temperature). This reduces the risk of growing harmful pathogens. Examiners ask this every year.

Common Mistake: Do NOT open the plate after incubation. Measure zones through the base of the plate using a ruler. Opening risks contaminating yourself and the environment.